

## **Supporting Material**

### **Thiol-Disulfide Exchange in Peptides Derived from Human Growth Hormone**

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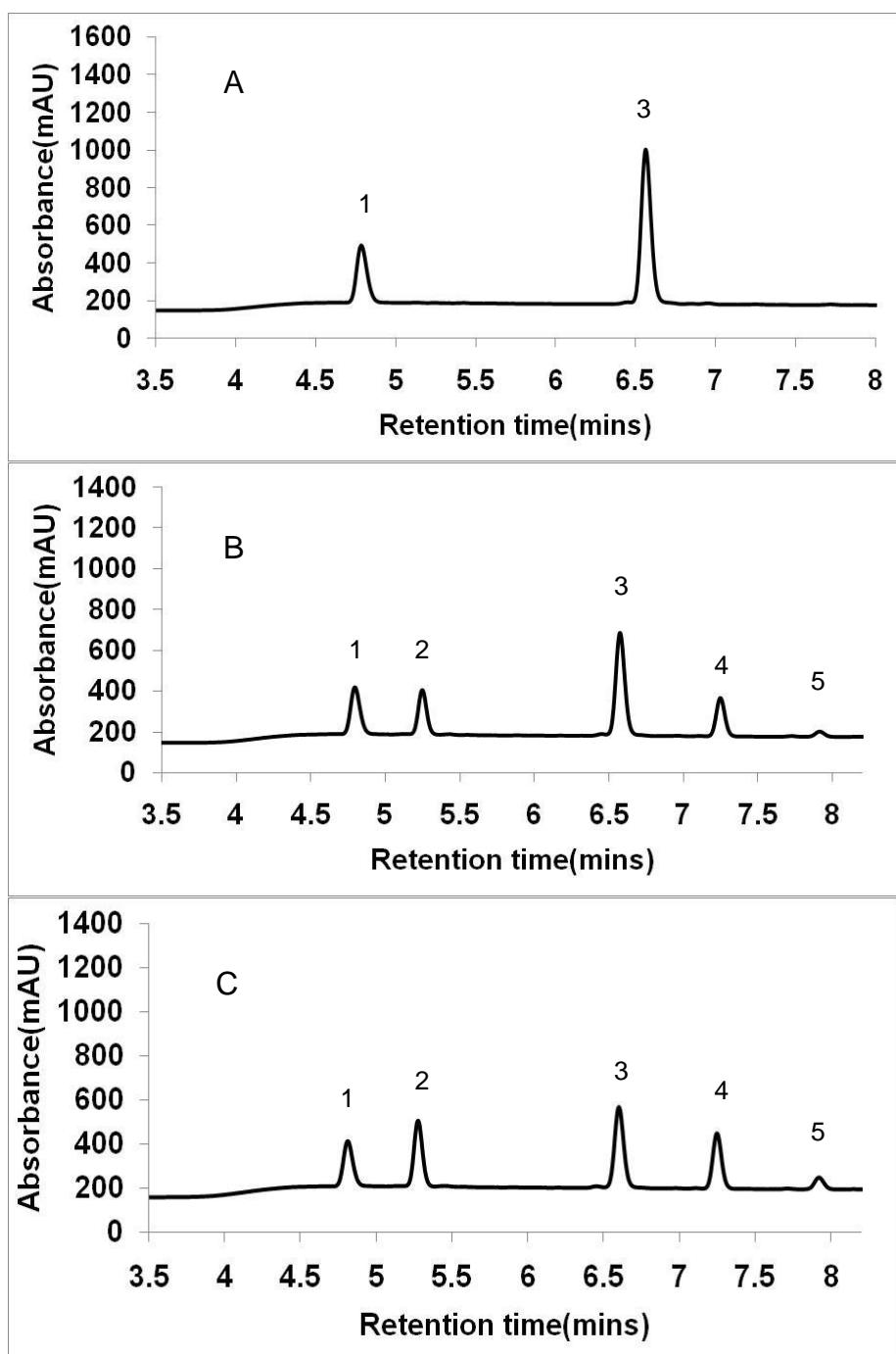
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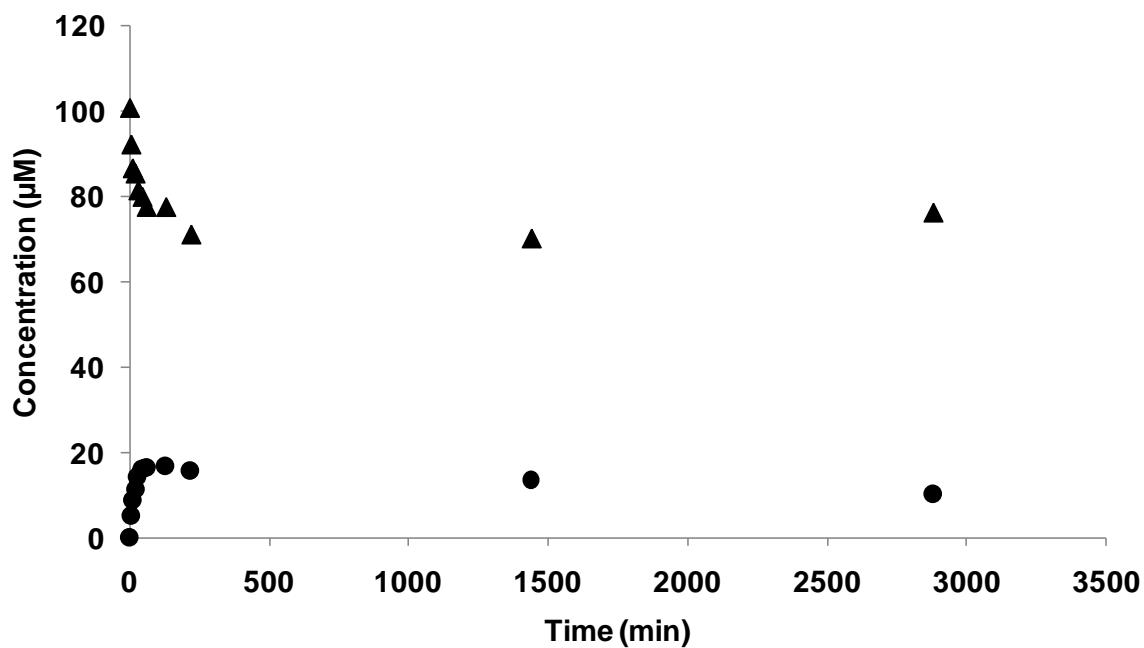
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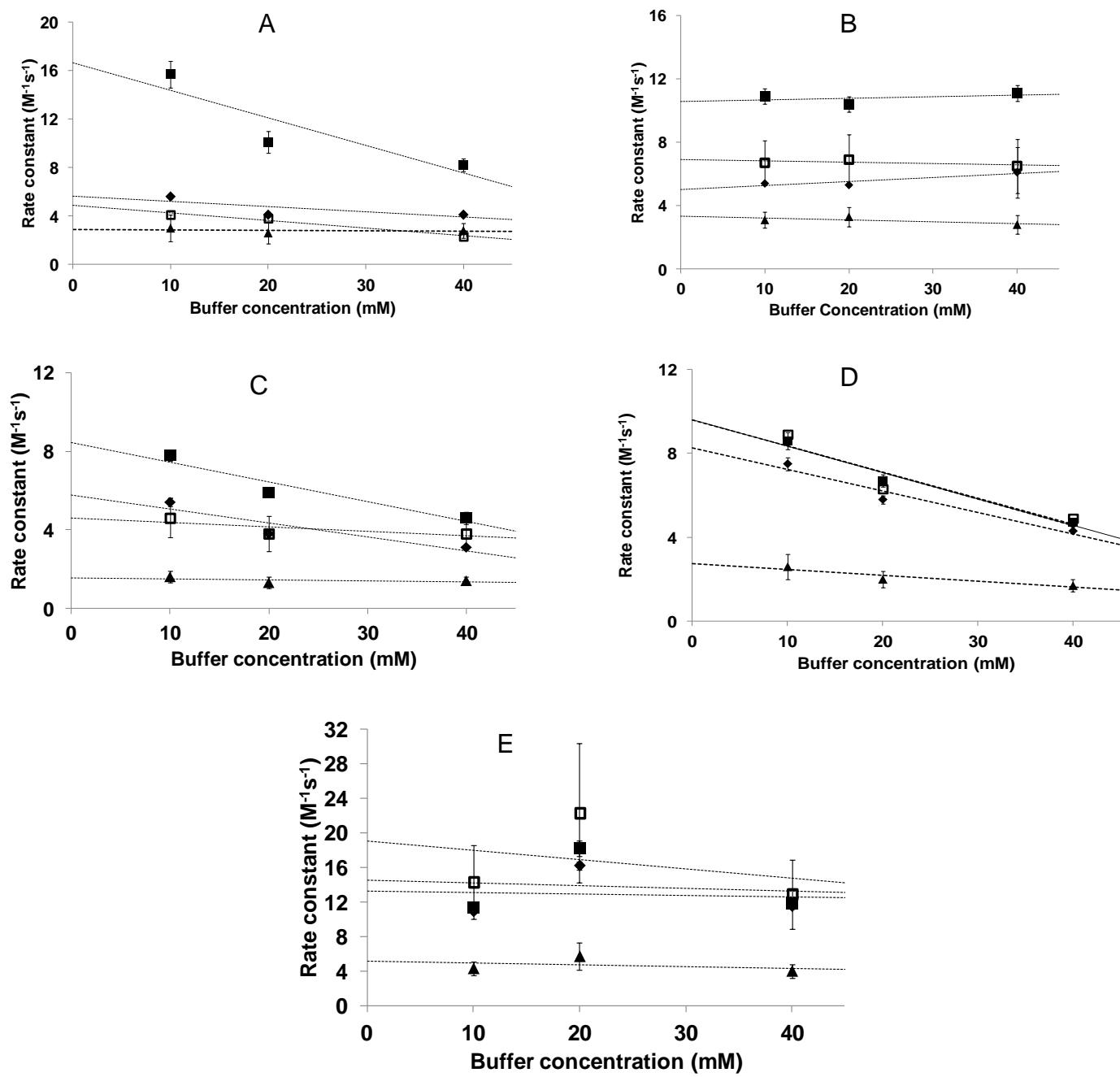
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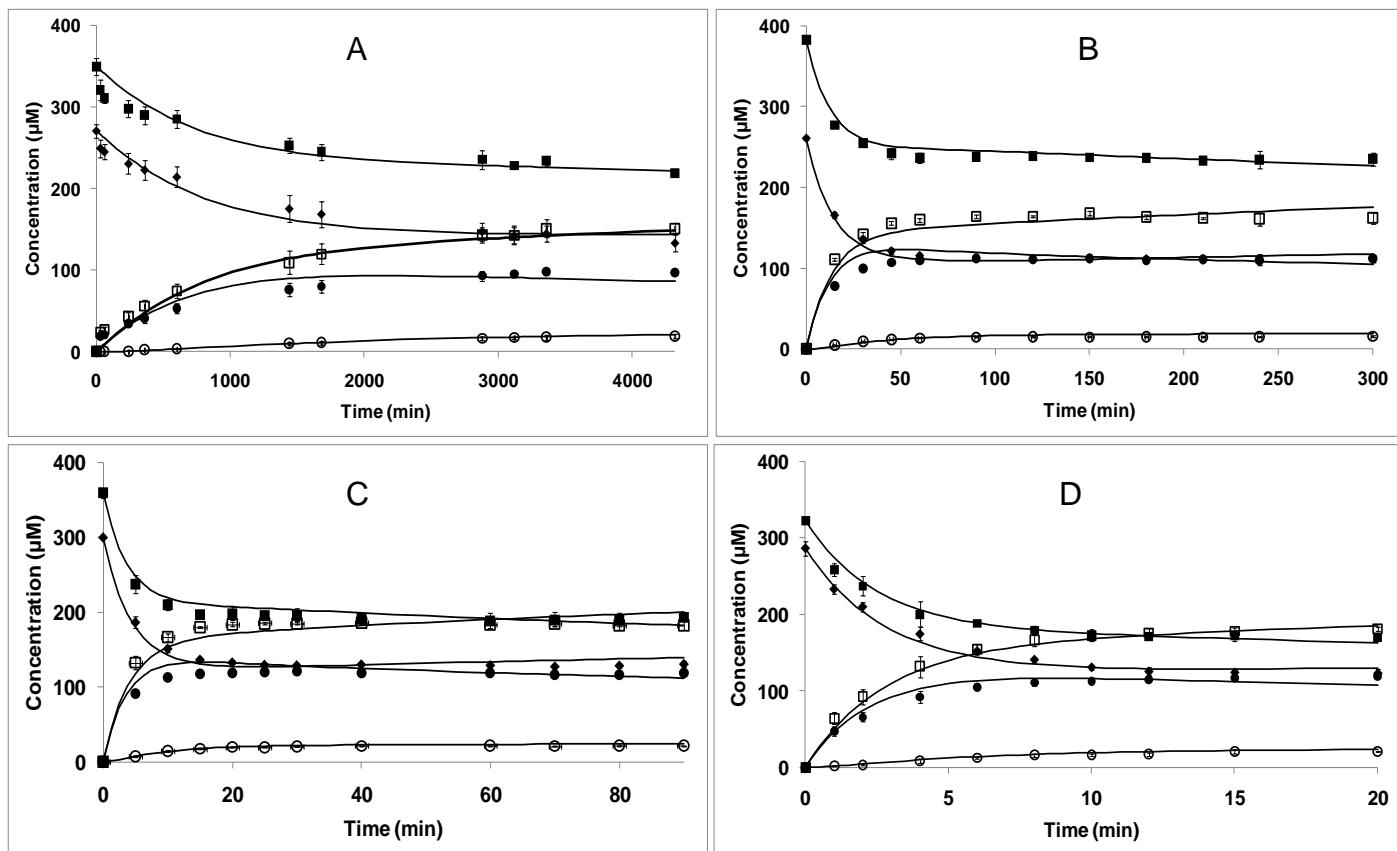
**Figure S1.** HPLC chromatograms (215 nm) at various times during the reaction of T20 with T20-T21 at pH 7.0, 10 mM phosphate buffer with 0.5 mM EDTA. A) 0 min, B) 180 min and C) 1440 min. Peak labels: 1) T20; 2) T20-T20; 3) T20-T21; 4) T21; 5) T21-T21.



**Figure S2.** Concentrations of cT20-T21 (▲) and rT20-T21 (●) for the reaction of cT20-T21 with 10 mM carbonate buffer, pH 9.0, and 0.08 M ionic strength (with EDTA and N<sub>2</sub> sparging) at 22 °C.



**Figure S3.** Rate constants vs. buffer concentration. Rate constants  $k_1$  (♦),  $k_2$  (■),  $k_3$  (▲) and  $k_4$  (□) were obtained from model fits to reaction scheme 1: A) pH 6.0, B) pH 7.0, C) pH 8.0, D) pH 9.0, and E) pH 10.0 at 0.08 M ionic strength, different buffer concentrations of 10, 20 and 40 mM (with EDTA and  $N_2$  sparging) and at 22 °C. Initial concentrations of peptides were: [T20] = 350  $\mu$ M; [T20-T21] = 250  $\mu$ M.



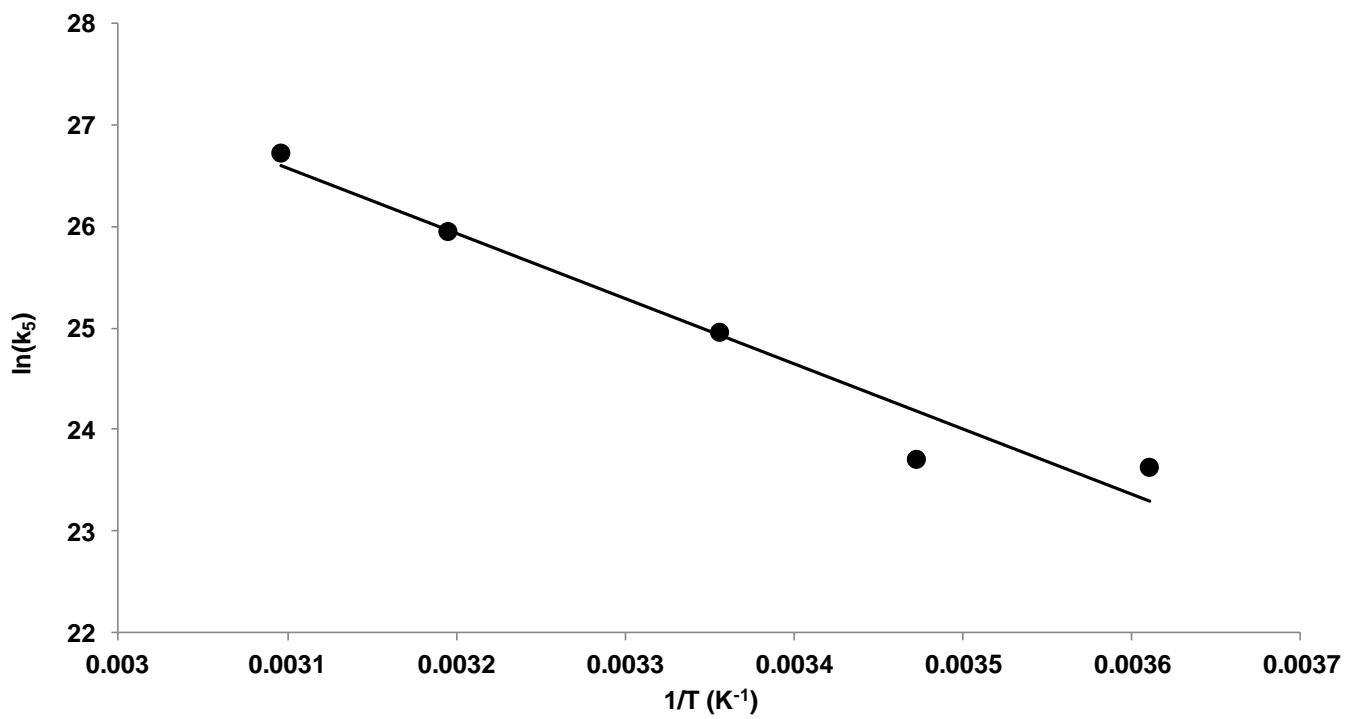
**Figure S4.** Kinetic plots at different pH for the reaction of T20 with T20-T21 at 22 °C. Symbols represent actual data points: T20 (■), T21 (●), T20-T20 (□), T21-T21 (○) and T20-T21 (◆). [T20] = 350  $\mu$ M; [T20-T21] = 250  $\mu$ M. Buffer conditions: pH 6.0-8.0 is with 10 mM phosphate buffer and pH 9.0-10.0 is with 10 mM carbonate buffer. Ionic strength (0.08 M) and EDTA (0.5 mM) were the same at all pH values. A) pH 6.0, B) pH 8.0, C) pH 9.0, and D) pH 10.0.

**Table S1.** Measured rate constants for all buffer concentrations for the reaction of T20 with T20-T21, 0.08 M ionic strength (with EDTA and N<sub>2</sub> sparging) at 22 °C.

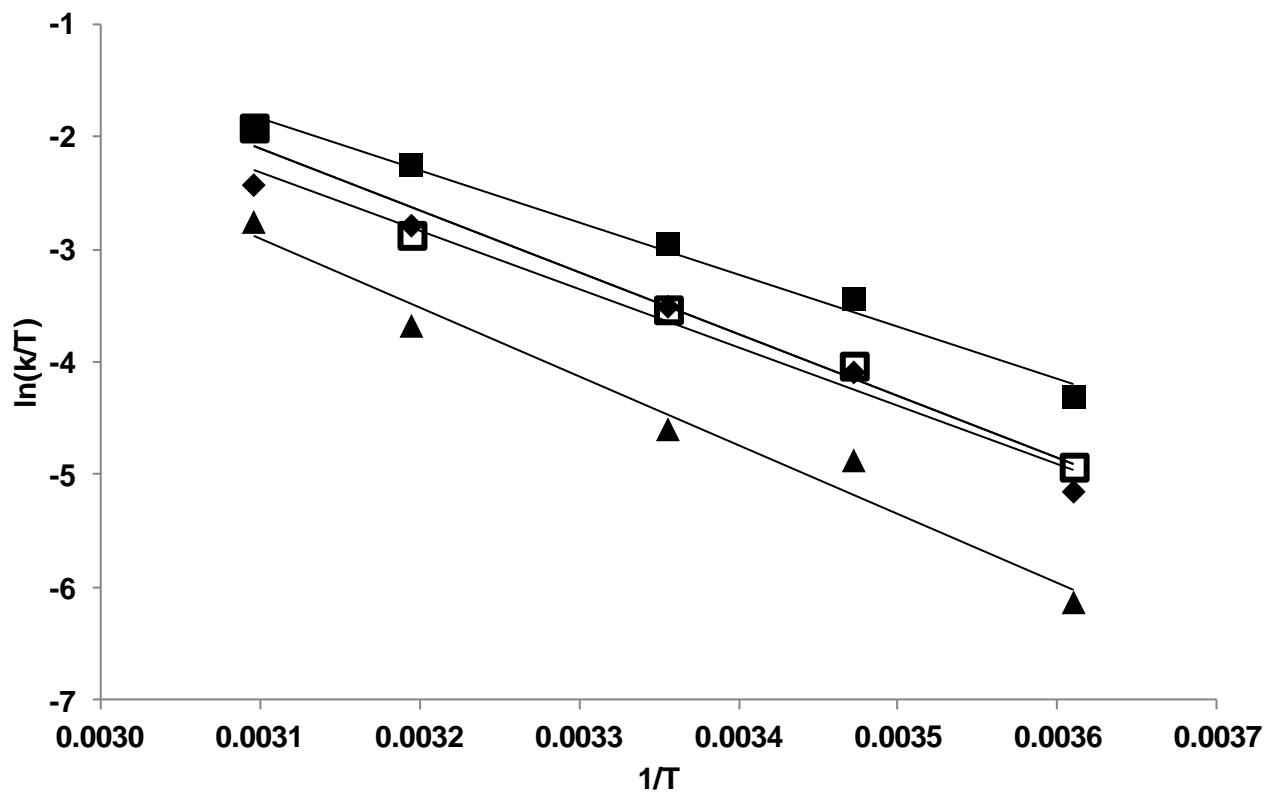
pH	Buffer concentration (mM)	k <sub>1</sub> (M <sup>-1</sup> s <sup>-1</sup> )	k <sub>2</sub> (M <sup>-1</sup> s <sup>-1</sup> )	k <sub>3</sub> (M <sup>-1</sup> s <sup>-1</sup> )	k <sub>4</sub> (M <sup>-1</sup> s <sup>-1</sup> )	k <sub>5</sub> (M <sup>-3</sup> s <sup>-1</sup> ) * 10 <sup>6</sup>
6.0	40	4.1±0.1	8.2±0.5	2.8±0.6	4.2±2.3	(163±11)*10 <sup>6</sup>
	20	4.1±0.2	10.1±0.9	2.6±0.9	4.4±3.8	(143±15)*10 <sup>6</sup>
	10	5.6±0.2	15.7±1.1	3.0±1.1	5.6±4.1	(147±14)*10 <sup>6</sup>
7.0	40	6.1±1.6	11.1±0.5	2.8±0.6	6.5±1.7	(223±17)*10 <sup>2</sup>
	20	5.3±0.1	10.4±0.5	3.3±0.6	6.9±1.6	(350±33)*10 <sup>2</sup>
	10	5.4±0.1	10.9±0.5	3.1±0.5	6.7±1.4	(333±28)*10 <sup>2</sup>
8.0	40	3.1±0.1	4.6±0.3	1.4±0.2	3.8±0.6	123±6
	20	3.8±0.1	5.9±0.2	1.3±0.3	3.8±0.9	80±4
	10	5.4±0.2	7.8±0.3	1.6±0.3	4.6±1.0	82±4
9.0	40	4.3±0.1	4.7±0.2	1.7±0.3	4.9±1.1	12±1
	20	5.8±0.2	6.7±0.3	2.0±0.4	6.3±1.5	13±1
	10	7.5±0.3	8.6±0.4	2.6±0.6	8.9±2.2	13±1
10.0	40	11.5±0.3	11.8±0.5	4.0±0.8	12.9±4.0	35±2
	20	16.2±0.5	18.2±0.9	5.7±1.6	22.3±8.1	37±2
	10	10.9±0.2	11.3±0.5	4.3±0.8	14.3±4.3	36±2

**Table S2.** Microscopic rate constants for the reaction of T20 and T20-T21 in the presence and absence of oxidation suppressants, 0.08 M ionic strength at 22 °C.

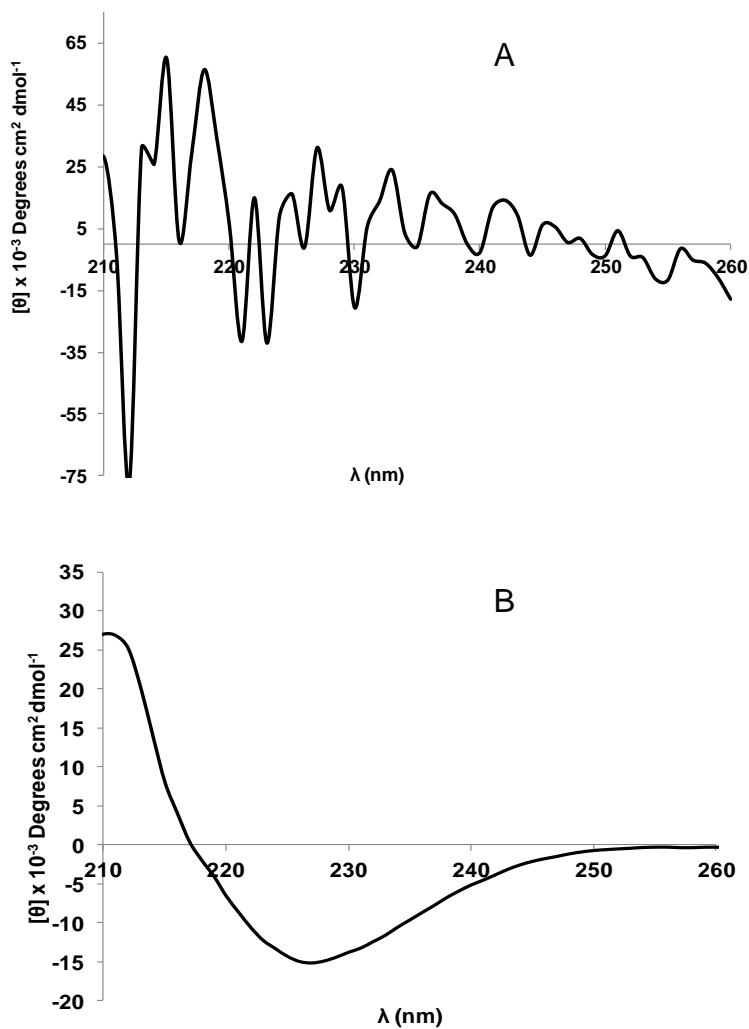
Reaction condition	pH	Buffer conc. (mM)	k <sub>1</sub> (M <sup>-1</sup> s <sup>-1</sup> )	k <sub>2</sub> (M <sup>-1</sup> s <sup>-1</sup> )	k <sub>3</sub> (M <sup>-1</sup> s <sup>-1</sup> )	k <sub>4</sub> (M <sup>-1</sup> s <sup>-1</sup> )	k <sub>5</sub> (M <sup>-3</sup> s <sup>-1</sup> )	k <sub>6</sub> (M <sup>-1</sup> s <sup>-1</sup> )
Without oxidation suppressants	7.0	10	5.7±0.2	9.6±0.4	1.8±0.4	5.6±1.6	(1100±60)*10 <sup>8</sup>	8.9±2.6
With oxidation suppressants	7.0	10	5.4±0.1	10.9±0.5	3.1±0.5	6.7±1.4	(300±30)*10 <sup>8</sup>	0.0



**Figure S5.** Arrhenius plot for microscopic rate constant  $k_5$  (●) for the reaction of T20 and T20-T21 (see Scheme 1). The reaction was monitored at pH 7.0, 10 mM buffer and 0.08 M ionic strength (with EDTA and N<sub>2</sub> sparging).



**Figure S6.** Eyring plot for the microscopic rate constants  $k_1$  (♦),  $k_2$  (■),  $k_3$  (▲) and  $k_4$  (□) for the reaction of T20 with T20-T21 at pH 7.0, 10 mM buffer and 0.08 M ionic strength (with EDTA and N<sub>2</sub> sparging).



**Figure S7.** Far-UV CD spectra for A) T20-T21 and B) cT20-T21. The far-UV CD spectrum for T20-T21 is representative of peptides T20, T21, T20-T20 and T20-T21.